Organellar proteomics: the prizes and pitfalls of opening the nuclear envelope

Minireview

Organellar proteomics: the prizes and pitfalls of opening the nuclear envelope

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Abstract

Proteomic studies have the potential to comprehensively define the composition of organelles but are limited by the organellar cross-contamination that arises during subcellular fractionation. Comparative proteomics of organellar subfractions can mitigate these problems, as demonstrated by a recent study involving the nuclear envelope.

Although decades have passed since most cellular organelles were initially characterized by microscopy and subcellular fractionation, a complete catalogue of the proteins in each organelle has yet to be obtained. Whereas genomics provides a list of potential proteins encoded by an organism's genome, data from proteomic analysis can provide the 'Rosetta stone' that allows assignment of specific proteins to different subcellular structures. The most informative proteomic analysis requires highly purified organelle (sub)fractions, yet subcellular fractionation is notorious for cross-contamination. Comparative analysis of organellar subfractions can potentially circumvent this problem by providing a rational basis for distinguishing *bona fide* organelle components from contaminants. Here, we scrutinize the use of such an approach to survey the integral membrane proteins of the nuclear envelope.

Organization of the nuclear envelope

The nuclear envelope is a double-membrane system, continuous with the endoplasmic reticulum (ER), that encloses the nuclear contents (Figure 1). It is perforated by nuclear pore complexes (NPCs), large supramolecular assemblies that mediate nucleo-cytoplasmic traffic [1,2]. In higher eukaryotes, the nuclear envelope is lined by a protein meshwork, the nuclear lamina, which is an attachment site for NPCs [3] and chromatin [4]. The outer nuclear membrane is biochemically similar to peripheral ER. In contrast, the inner nuclear membrane (INM) contains a set of unique integral membrane proteins, many of which bind to lamins (the predominant proteins of the nuclear lamina) and/or to chromatin [5].

A quarter of a century of biochemical, cell biological, and genetic approaches has identified several integral INM proteins in higher eukaryotes, including lamin-B receptor (LBR) [6], lamina-associated polypeptides (LAPs) 1 and 2 [7], MAN1 [8], nurim [9], and emerin [10]. Most of these proteins and several novel components have now been detected in a single proteomics study of nuclear envelopes [11].

Proteomics of the nuclear envelope: the prizes

Many methods have been developed to isolate nuclear and nuclear-envelope fractions from eukaryotic cells. These fractions are invariably contaminated by other cytoplasmic organelles and filaments, however. Moreover, the close interactions between the NPCs, nuclear lamina, INM and chromatin make it impossible to cleanly separate these components. Proteomics, which couples mass-spectrometric analysis of proteins with mining of the protein databases derived from genomics, can potentially identify most proteins in a nuclear envelope fraction, but it cannot distinguish nuclear envelope components from contaminants.

Dreger and colleagues [11] used comparative proteomics of different nuclear envelope subfractions isolated from cultured

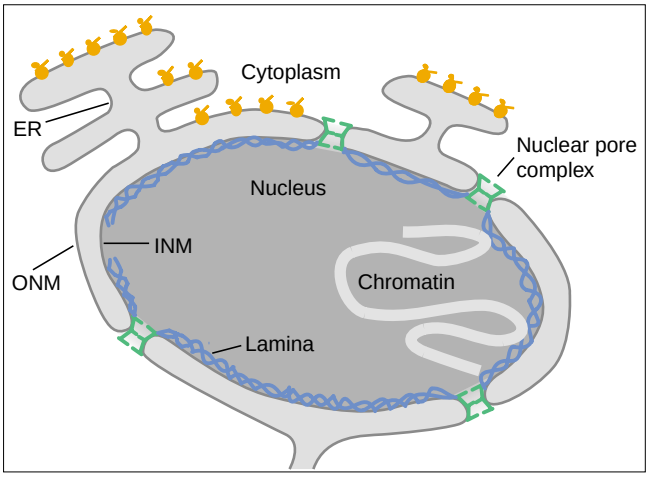

Figure 1
Schematic of the nuclear envelope. The outer nuclear membrane (ONM) and inner nuclear membrane (INM) form a double-membrane system that separates nuclear contents from the cytoplasm. Nuclear pore complexes bridge this system and regulate nucleo-cytoplasmic exchange of macromolecules. Further attachments to many other proteins make purification of individual compartments difficult.

neuroblastoma cells to identify integral proteins of the INM. They took advantage of previous findings that the nuclear lamina is insoluble in both nonionic detergent and salt, and that many integral proteins of the INM remain associated with the lamina after detergent or salt extraction. They therefore separated proteins from three different nuclear envelope subfractions on two-dimensional gels, excised protein spots from the gels, cleaved the proteins within each spot with protease, and analyzed the resulting peptides by MALDI-TOF (matrix-assisted laser-desorption/ionization time-of-flight) mass spectrometry. In one extraction, nuclear envelopes were treated with the detergent Triton X-100 to solubilize proteins of the outer nuclear membrane and ER. The Triton X-100 pellet ('detergent-resistant' in Table 1), which contains the nuclear lamina, is enriched in lamin-binding INM proteins. A second pellet was obtained by salt extraction of nuclear envelopes ('salt-resistant'; Table 1) and is expected to be similarly enriched in the lamina, but with different contaminants removed. A third extraction involved treatment of nuclear envelopes with a urea/carbonate solution and yields a pellet ('chaotrope-resistant'; Table 1) containing integral membrane proteins of the INM, outer nuclear membrane and ER (plus contaminating organelles). On the basis of the logic discussed above, integral membrane proteins (chaotrope pellet) that were also found in the detergent and salt pellets were good candidates for novel INM proteins.

This analysis [11] identified most previously characterized INM proteins, as well as four novel integral proteins. Two of the novel four were splice variants of the lamina-associated protein LAP2, previously observed only as mRNAs [12]. The identification of unique peptides, including some that overlap predicted splice junctions between exons, confirmed the presence of three previously observed (α, β, γ) and two novel (δ, ε) LAP2 isoforms. The remaining two LAP2 isoforms that had been identified as mRNAs (ζ, β′) were not observed (although all LAP2 isoforms contain chromatin and/or lamin-binding domains that predict their targeting to the nuclear envelope). LAP2α, a splice variant lacking a transmembrane domain, was absent from the chaotrope-resistant fraction, supporting the logic of the comparative approach. The third novel INM protein to be identified was a homolog of Unc84A, a *Caenorhabditis elegans* protein previously localized to the nuclear envelope [13]. Unc84A is important for nuclear migration and is conserved from fission yeast to humans. The fourth protein, LUMA, was completely novel and includes no known domains and so has no predicted functions.

Pitfalls of proteomic analysis

Proteomics may miss some constituents of subcellular fractions. Dreger and colleagues [11] did not detect LAP1, although it has been observed in a variety of mammalian cell types [7]. This could result from its loss during fractionation, its absence from the neuroblastoma line examined, or other technical problems (for example, only about 75% of the protein spots seen on the two-dimensional gel yielded assignments by mass spectrometry). Low abundance is also a potential problem. Whereas lamins are present at millions of copies per cell, previously characterized INM proteins are

Table 1

Integral membrane proteins recovered in various cellular fractions by Dreger *et al.* [11]

	Detergent-resistant	Salt-resistant	Chaotrope-resistant	Newly identified by this study
Emerin		+	+	
LAP1				
LAP2α	+	+		
LAP2β	+	+	+	
LAP2β′				
LAP2δ	+	+	+	+
LAP2ε	+	+	+	+
LAP2γ	+	+	+	
LAP2ζ				
LBR	+			
LUMA	+		+	+
MAN-1	+	+	+	
Nurim	+	+	+	
Unc84 homolog	+	+	+	+

Details of how the fractions were obtained are given in the text.

far less abundant. Notwithstanding the high sensitivity of mass spectrometry, minor proteins may go undetected.

Just as local dialect modifications can limit some translations using the Rosetta stone, atypical protein behaviors can limit comparative proteomic approaches. Each comparative approach is tailored by knowledge of the fractionation behavior of previously characterized components of an organelle. Thus, in comparing the proteins appearing in the three nuclear envelope fractions, Dreger and colleagues [11] found that all well characterized intranuclear proteins were absent from the chaotrope-resistant fraction, and most known INM proteins were in all three fractions. Two known INM proteins were atypical, however. Emerin, which is known to bind lamins, was absent from the detergent-resistant fraction, and LBR, the sequence of which includes seven transmembrane segments, was detected only in the detergent-resistant fraction and not in the chaotrope- or salt-resistant fractions (Table 1). Three of the novel proteins identified by the study were in all three fractions, but LUMA was absent from the salt-resistant fraction. Thus, LUMA would have been missed if a requirement that novel INM proteins appear in all three fractions had been enforced.

Atypical behavior of contaminants can also limit the effectiveness of comparative approaches. In the Dreger study [11], some known proteins with clearly cytoplasmic localizations and functions also appeared in all three pellets, including mitochondrial proteins (F1-ATP-synthase α) and cytoskeletal ones (actin and tubulin). This underscores the need to complement the proteomic identification of new organellar components with other approaches, including direct localization. Thus, to ensure that LUMA and the Unc84A homolog were not contaminants, their cDNAs were isolated, epitope-tagged, and transfected into COS7 cells, to confirm nuclear-envelope targeting [11].

Rout and colleagues, when analyzing the yeast NPC by proteomics [14], used the additional criterion of enrichment with the NPC to distinguish contaminants. Candidate proteins from an NPC-enriched fraction were epitope-tagged at their genomic loci. Only when the majority of tagged protein was found to reside at the NPC (by immunogold electron microscopy, immunofluorescence microscopy, and co-fractionation with known NPC proteins) was it considered a true NPC component. Some proteins have multiple cellular localizations, however, and this criterion eliminated, for example, the Sec13 protein, which is involved in protein translocation across the ER membrane yet has a second function at the NPC [15].

Opening the envelope

Even with the four novel INM proteins demonstrated by the Dreger study [11], we predict that many INM proteins remain to be discovered; some of these could be amongst the 25% of protein spots that eluded analysis. The nuclear envelope also probably includes cell-type specific INM proteins. For example, Unc84A appears in all *C. elegans* tissues, but Unc83, an INM protein that interacts with Unc84A, appears in only a subset [16]. Nuclear envelope proteome analyses of different tissues may uncover a mammalian Unc83 homolog and other tissue-specific INM proteins. Indeed, a novel tissue-specific INM protein was just discovered. This protein, named Myne-1, binds lamins and appears specifically in myocytes [17].

There are many ways that organellar proteomics can be modified to yield additional information. Applying proteomics directly to complex protein mixtures [18], rather than restricting analysis to the proteins recovered from gels, should increase the number of proteins detected. Moreover, this method could estimate the relative amounts of different proteins in a subcellular compartment by determining the relative peptide quantities after protease cleavage. Post-translationally modified peptides could be identified by comparing peptide profiles with or without treatments that remove the modifications (for example, chemical removal of sugars [19]); and a combination of this type of approach with relative quantitation could provide information on the proportion of a protein in an organelle that is modified. By analysis of complex mixtures, the protein profiles of organelles in different functional states or extracted with different ionic strengths can be compared more comprehensively and rapidly than with classical approaches.

In the past two years, organellar proteomics has also profiled mitochondrial [20], chloroplast [21], and nucleolar [22] proteomes, uncovered minor Golgi proteins [23], and compared functional states of the Golgi [24]. The speed of proteomics can produce petabytes of data far faster than we can analyze them. It thereby allows a wider range of comparative analyses than has so far been possible. The roads opened by comparative proteomics will one day provide a complete map of all the cellular proteins in each organelle in each tissue at each stage of development. Through comparison of this map with datasets generated from people with particular diseases, proteomics provides a new way to identify the causes of disease. The opening of the nuclear envelope by proteomics has already identified new INM proteins and produced a catalogue of yeast NPC proteins. Much more is likely to come from future analyses.

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